Remarks

Amendments to the Claims and Specification

The claims are amended to remove multiple dependencies. New claim 41-43 is supported by original claim 37. New claims 42 and 43 are supported by original claim 38.

The specification is amended to delete hyperlinks and to insert sequence identifiers. The amendments add no new matter to the specification.

Formal Sequence Listing

A paper and a computer readable form of a formal sequence listing accompany this amendment. I believe the sequence contents of the paper and computer readable forms are identical.

The formal sequence listing adds no new matter to the specification. It contains the primer sequences disclosed at page 13 as SEQ ID NOS:1-6. SEQ ID NOS:7-22 are the sequences present in the informal sequence listing as SEQ ID NOS:1-16, respectively.

Respectfully submitted,

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Appendix 1. Version of Amended Paragraphs with Markings to Show Changes Made

On page 1, in the title:

[POLYSATURATED] POLYUNSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS

Page 3, first full paragraph:

In order to identify genes encoding PUFA elongases, it is necessary to study systems in which the synthesis of PUFAs is well documented; a good example of this is the model animal system *C. elegans*, a small free-living worm (Tanaka *et al.*, (1996), *Lipids* 31, 1173-78). *C. elegans*, like most other animals, and in contrast to higher plants, synthesizes PUFAs such as arachidonic acid (AA; 20:4 $\Delta^{5, 8, 11, 14}$) as precursors to a class of molecules known as the eicosanoids, which in turn serve as precursors for compounds such as prostaglandins and leucotriens (Horrobin, (1990), *Review in Contemp* [*Pharmacotherpy*] *Pharmacotherapy*, 1:1-45). The presence of AA and other long chain polyunsaturated fatty acids in *C. elegans* is well documented (Tanaka *et al.*, (1996), *Lipids* 1, 1173-1178). The complete sequence of the nematode's genome is now publicly available (*The C. elegans cosortium, 1998, Science 282, 2012-2018.* See the database at the website identified with the URL file type, www host server, domain name sanger.ac.uk and following the path from "Projects" to "C_elegans" to "blast_server.shtml" [*Database at http://www.sanger.ac.uk/Projects/C_elegans/blast_server.shtml)*].

Page 7, line 18 to page 8, line 21:

Initially the *C. elegans* databases were searched for any sequences which showed low levels of homology to yeast ELO genes (*ELO2* and *ELO3*) using the TBLASTN programme. A similar search was carried out using short (20 to 50 amino acid) stretches of ELO genes which were conserved amongst the three

ELO polypeptide sequences. *C. elegans* sequences which were identified by this method were then used themselves as search probes, to identify any related *C. elegans* genes which the initial search with the yeast sequences failed to identify. This was necessary because the level of homology between the yeast ELO genes and any worm genes is always low (see BLAST scores later). To allow for a more sensitive search of worm sequences, a novel approach was adopted to circumvent the major drawback with searches using the BLAST programmes, namely that the search string (i.e. the input search motif) must be longer than 15 characters for the algorithm to work. Thus, if it was desired to search for a short motif (like a histidine box), then the BLAST programme would not be capable of doing this. A complete list of all the predicted ORFs present in the *C. elegans* genome exists as a database called Wormpep, which is freely available from the Sanger WWW site identified with the URL address http file type, www host server, domain name sanger.ac.uk and following the path from "Projects" to "C_elegans" to "webace_front_end.shtml" [(http://www.sanger.ac.uk/Projects/C_elegans/webace_front_end.shtml)]. The latest version of Wormpep was down loaded to the hard disc of a Pentium PC, and re-formatted as a Microsoft Word6 document, resulting in a document of about 3,500 pages. This was then searched using the "Search & Replace" function of Word6, which also allows for the introduction of "wildcard" characters into the search motif. So, for example, it is possible to search both for the short text string HPGG, which would identify any predicted worm ORF present in the Wormpep 3,500 page document containing this motif, or alternatively search with HPGX (where X is a wild card character). Clearly, such (manual) searches of a 3,500 page document are extremely time-consuming and demanding, also requiring visual inspection of each and every identified ORF. For example, searching with a motif such as HXXHH identifies in excess of 300 different ORFs. However, by using a number of different short search strings (as outlined below), and combining these with other methods for identifying putative elongase enzymes, a number of candidate ORFs have been identified.

Page 8, line 23 to page 9, line 3:

As a negative control, to demonstrate that the FAE1 gene sequence was unlikely to provide a useful search sequence in the identification of *C. elegans* sequences encoding for PUFA elongases, the GenBank databases [(http://www.ncbi.nlm.nih.gov/Web/Search/index.html)] identified with the URL address http file type, www host server, domain name "ncbi.nlm.nih.gov" and following the path from Web to Search to index.html were searched using the *Arabidopsis* FAE1 polypeptide sequence ot identify related genes or expressed sequence transcripts (ESTs). GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (*Nucleic Acid Research* (1998) 26, 1-7). There are approximately 2,162,000,000 bases in 3,044,000 sequence records as of December 1998. The search was carried out using the BLAST2 (Basic Local Alignment Search Tool) algorithm (Altschul *et al.*, (1990) *J Mol Biol* 215, 403, 410). Although a number of plant ORFs and ESTs were reported as being related, o animal sequences were identified by this search, confirming the observation that FAE1 was unlikely to be a suitable candidate as a search template for PUFA elongases.

Page 9, line 5:

Using the three yeast fatty acid elongase sequences (ELO 1, 2, 3) as probes, a number of putative ORFs in the DNA of *C. elegans*-derived cosmid sequences which form the *C. elegans* genomic sequence database was identified. Moreover, an extensive and time-consuming search of a downloaded copy of the WormPep database identified with the URL address ftp file type, ftp host server, domain name sanger.ac.uk, following the path from "pub" to "databases" to "wormpep" [(ftp://ftp.sanger.ac.uk/pub/databases/wormpep)] using manual search strings in MSWord 6, identified a number of *C. elegans* ORFs which contained presumptive histidine boxes. Wormpep contains predicted proteins from the *Caenorhabditis elgans* genome sequence project, which is

carried out jointly by the Sanger Centre in Cambridge, UK and Genome Sequencing Center in St. Louis, USA. The current Wormpep database, Wormpep 16, contains 16,332 protein sequences (7,120,115 residues). Search strings used included [HXXHH], [HXXXHH], [QXXHH] and [YHH]. Comparison of the data from the two different searches indicated a small (<10) number of putative ORFs as candidate elongases. The histidine box motifs are [shown in bold in SEQ ID 9 to 16] located at amino acids 162-166 of SEQ ID NO:15, amino acids 186-190 of SEQ ID NO:16, amino acids 145-150 of SEQ ID NO:17, amino acids 147-151 of SEQ ID NO:18, amino acids 141-145 of SEQ ID NO:19, amino acids 177-181 of SEQ ID NO:20, amino acids 155-159 of SEQ ID NO:21, and amino acids 233-237 of SEQ ID NO:22.

Page 10, line 7:

Since the inventors had previously observed that *C. elegans* genes involved in the synthesis of PUFA may exist in tandem (for example the Δ5 and Δ6 desaturases required for AA and GLA synthesis, respectively, are <1 kB apart on chromosome IV (Michaelson et al., (1998), *FEBS Letts* **439**, 215-218), the positions of the putative *C. elegans* elongase ORFs were determined using the Sanger Centre's WebAce *C. elegans* server identified with the URL address http filetype, www host server, domain name sanger.ac.uk and following the path from "Projects" to C_elegans" to "webac_front_ends.shtml" [(http://www.sanger.ac.uk/Projects/C_elegans/webac_front_ends.shtml).]. This indicated that two pairs of putative elongases were in close proximity to each other on the *C. elegans* chromosome IV.

Page 13, line 2:

Putative elongase sequences F56H11.4 and F41H10.8 were cloned by PCR into the pYES2 vector (Invitrogen). A *C. elegans* mixed stage cDNA library was used as a PCR template. F56H11.4 was amplified using primers:

56h114.for 5'-GCGGGTACCATGGCTCAGCATCCGCTC-3' (SEQ ID NO:1) and;

56h114.rev 5'-GCGGGATCCTTAGTTGTTCTTCTTCTT-3' (SEQ ID NO:2).

F41H10.8 was amplified using primers:

41h108.for 5'-GCGGGTACCATGCCACAGGGAGAAGTC-3' (SEQ ID NO:3) and;

416h108.rev 5'-GCGGGATCCTTATTCAATTTTTCTTTT-3' (SEQ ID NO:4).

Page 13, line 13:

An ORF encoding the *Mortierella alpina* Δ^5-fatty acid desaturase (Michaelson, L.V., et al. (1998) *J. Biol. Chem.*, **273**, 19055-19059) was amplified using primers:

Mad5.for 5'-GCGAATTCACCATGGGTACGGACCAAGGA-3' (SEQ ID NO:5) and;

Mad5.rev 5'-GCGGAGCTCCTACTCTTCCTTGGGACG-3' (SEQ ID NO:6).

Appendix 2. Version of Amended Claims with Markings to Show Changes Made

1. (Amended) An isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase [as herein defined] having a function of extending a chain length of an 18 carbon PUFA to 20 carbons in length.

3. (Amended) A polypeptide according to claim 1 [or claim 2] wherein the polypeptide [has at least] comprises a portion of the amino acid sequence shown in SEQ ID NO:15 or a variant [variants] thereof.

7. (Amended) A polypeptide according to [any preceding] claim 1 wherein the polypeptide sequence includes a sequence motif responsible for Endoplasmic Reticulum (ER)-retention.

8. (Amended) A polypeptide according to [any preceding] claim 1 wherein the polypeptide is capable of elongating palmitoleic acid (PA; $16:1\Delta^{9}$) to vacceric acid (VA; $18:1\Delta^{11}$).

9. (Amended) A polypeptide according to [any preceding] claim 1 wherein the polypeptide is [from] an animal polypeptide.

16. (Amended) [A] An isolated DNA [sequence] molecule encoding a polypeptide according to [any preceding] claim 1.

17. (Amended) [A] A DNA [sequence] molecule according to claim 16 wherein the DNA molecule comprises the sequence shown in SEQ ID NO:7 or variants of that sequence due to base substitutions, deletions, and/or additions.

18. (Amended) An engineered organism engineered to express a polypeptide according to [any one of claims] claim 1.

21. (Amended) An engineered organism containing a synthetic pathway for the production of a polypeptide according to [any one of claims] claim 1.

23. (Amended) An engineered organism according to claim 21 [or 22] wherein the pathway includes Δ^6-fatty acid desaturase.

24. (Amended) An engineered organism according to [any one of claims] claim 21 [to 23] wherein the [animal] organism is a lower eukaryote.

27. (Amended) A transgenic plant engineered to express a polypeptide according to [any one of claims] claim1 [to 15].

28. (Amended) A transgenic plant containing a DNA [sequence] molecule according to claim 16 [or 17].

29. (Amended) A method of producing a PUFA comprising carrying out an elongase reaction catalysed by a polypeptide according to [any one of claims] claim 1 [to 15].

32. (Amended) A PUFA produced by a method according to [any one of claims] claim 29 [to 31].

35. (Amended) A pharmaceutical composition comprising a polypeptide according to [any one of claims] claim 1 [to 15].

37. (Amended) A pharmaceutical composition according to claim 35 [or claim 36] wherein the composition comprises a pharmaceutically-acceptable diluent, carrier, excipient or extender.

38. (Amended) A method of elevating the PUFA levels of an animal or a plant comprising the step of [by] supplying to the animal or plant a polypeptide according to [any of claims] claim 1 [to 15, a DNA sequence according to claim 16 or 17, a foodstuff according to claim 33, a dietary supplement according to claim 34, a pharmaceutical composition according to any of

claims 35 to 37 or a PUFA according to claim 32].

39. (Amended) A method [of treatment] according to claim 38 wherein the animal is a mammal.

40. (Amended) A method [of treatment] according to claim 39 wherein the mammal is a human.